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(54) Title: A TRUNCATED INTERLEUKIN-1 RECEPTOR GENE FOR THE TREATMENT OF ARTHRITIS (57) Abstract The subject invention concerns a method of using <i>in vivo</i> a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing interleukin-1 which includes employing recombinant techniques to produce a cell line under the control of a suitable eukaryotic promoter having the gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor; and initiating transfection of DNA of the gene by introducing viral particles obtained from the cell line directly into synovial cells lining a joint space of a mammalian host. Alternatively, synovial cells from the patient's joint may be transduced with the retroviral vector carrying the therapeutic gene and a selectable marker for selection of only transduced cells, and the now therapeutic autologous cells may be introduced back into the joint by transplantation. Additionally, a method of preparing a gene encoding an extracellular interleukin-1 receptor binding domain of an interleukin-1 that is capable of binding to and neutralizing interleukin-1 is disclosed. A compound for parenteral administration to a patient in prophylactically or therapeutically effective amounts containing the gene of the invention and a suitable pharmaceutical carrier is also provided.		

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⁺ Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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A TRUNCATED INTERLEUKIN-1 RECEPTOR GENE FOR THE TREATMENT OF ARTHRITIS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method of using a gene encoding a truncated interleukin-1 receptor to resist the deleterious pathological changes associated with arthritis. More specifically, this invention provides a method wherein a gene coding for an extracellular interleukin-1 binding domain of an interleukin-1 receptor is introduced into synovial cells of a mammalian host in vivo for neutralizing the destructive activity of interleukin-1 upon cartilage and other soft tissues. As an alternative, the patients own cells are transduced in vitro and introduced back into the affected joint, using surgical transplantation procedures.

Brief Description of the Prior Art

Arthritis involves inflammation of a joint that is usually accompanied by pain and frequently changes in structure. Arthritis may result from or be associated with a number of conditions including infection, immunological disturbances, trauma and degenerative joint diseases such as, for example, osteoarthritis. The biochemistry of cartilage degradation in joints and cellular changes have received considerable investigation.

In a healthy joint, cells in cartilage (chondrocytes) and the surrounding synovium (synoviocytes) are in a resting state. In this resting state, these cells secrete basal levels of prostaglandin E₂ and various neutral proteinases, such as, for example, collagenase, gelatinase and stromelysin, with the ability to degrade cartilage. During the development of an arthritic condition, these

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cells become activated. In the activated state, synoviocytes and chondrocytes synthesize and secrete large amounts of prostaglandin E₂ and neutral proteinases.

In efforts to identify pathophysiologically relevant cell activators, it has been known that the cytokine interleukin-1 activates chondrocytes and synoviocytes and induces cartilage breakdown in vitro and in vivo. Additionally, interleukin-1 is a growth factor for synoviocytes and promotes their synthesis of matrix, two properties suggesting the involvement of interleukin-1 in the synovial hypertrophy that accompanies arthritis. In contrast, interleukin-1 inhibits cartilaginous matrix synthesis by chondrocytes, thereby suppressing repair of cartilage. Interleukin-1 also induces bone resorption and thus may account for the loss of bone density seen in rheumatoid arthritis. Interleukin-1 is inflammatory, serves as a growth factor for lymphocytes, is a chemotactic factor and a possible activator of polymorphonuclear leukocytes (PMNs). When present in a sufficient concentration, interleukin-1 may cause fever, muscle wasting and sleepiness.

The major source of interleukin-1 in the joint is the synovium. Interleukin-1 is secreted by the resident synoviocytes, which are joined under inflammatory conditions by macrophages and other white blood cells.

Much attention has been devoted to the development of a class of agents identified as the "Non-Steroidal Anti-Inflammatory Drugs" (hereinafter "NSAIDs"). The NSAIDs inhibit cartilage synthesis and repair and control inflammation. The mechanism of action of the NSAIDs appears to be associated principally with the inhibition of prostaglandin synthesis in body tissues. Most of this development has involved the synthesis of better inhibitors of cyclo-

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oxygenase, a key enzyme that catalyzes the formation of prostaglandin precursors (endoperoxides) from arachidonic acid. The anti-inflammatory effect of the NSAIDs is thought to be due in part to inhibition of prostaglandin synthesis and release during inflammation. Prostaglandins are also believed to play a role in modulating the rate and extent of leukocyte infiltration during inflammation. The NSAIDs include, such as, for example, acetylsalicylic acid (aspirin), fenoprofen calcium (Nalfon® Pulvules®, Dista Products Company), ibuprofen (Motrin®, The Upjohn Company), and indomethacin (Indocin®, Merck, Sharp & Dohme).

In contrast, the studies upon which the present invention is based show that production of the various neutral proteinases with the ability to degrade cartilage occurs even if prostaglandin synthesis is completely blocked.

It has been shown that genetic material can be introduced into mammalian cells by chemical or biologic means. Moreover, the introduced genetic material can be expressed so that high levels of a specific protein can be synthesized by the host cell. Cells retaining the introduced genetic material may include an antibiotic resistance gene thus providing a selectable marker for preferential growth of the transduced cell in the presence of the corresponding antibiotic. Chemical compounds for inhibiting the production of interleukin-1 are also known.

U.S. Patent No. 4,778,806 discloses a method of inhibiting the production of interleukin-1 by monocytes and/or macrophages in a human by administering through the parenteral route a 2-2'-[1,3-propan-2-onediyl-bis (thio)] bis-1 H-imidazole or a pharmaceutically acceptable salt thereof. This patent discloses a chemical compound for inhibiting the production of interleukin-1. By contrast, in

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the present invention, gene therapy is employed that is capable of binding to and neutralizing interleukin-1.

U.S. Patent No. 4,780,470 discloses a method of inhibiting the production of interleukin-1 by monocytes in a human by administering a 4,5-diaryl-2 (substituted) imidazole. This patent also discloses a chemical compound for inhibiting the production of interleukin-1.

U.S. Patent No. 4,794,114 discloses a method of inhibiting the 5-lipoxygenase pathway in a human by administering a diaryl-substituted imidazole fused to a thiazole, pyrrolidine or piperidine ring or a pharmaceutically acceptable salt thereof. This patent also discloses a chemical compound for inhibiting the production of interleukin-1.

U.S. Patent No. 4,870,101 discloses a method for inhibiting the release of interleukin-1 and for alleviating interleukin-1 mediated conditions by administering an effective amount of a pharmaceutically acceptable anti-oxidant compound such as disulfiram, tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl) propionyloxy methyl] methane or 2,4-di-isobutyl-6-(N,N-dimethylamino methyl)-phenol. This patent discloses a chemical compound for inhibiting the release of interleukin-1.

U.S. Patent No. 4,816,436 discloses a process for the use of interleukin-1 as an anti-arthritic agent. This patent states that interleukin-1, in association with a pharmaceutical carrier, may be administered by intra-articular injection for the treatment of arthritis or inflammation. In contrast, the present invention discloses a method of using and preparing a gene that is capable of binding to and neutralizing interleukin-1 as a method of resisting arthritis.

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U.S. Patent No. 4,935,343 discloses an immunoassay method for the detection of interleukin-1 β that employs a monoclonal antibody that binds to interleukin-1 β but does not bind to interleukin-1 α . This patent discloses that the monoclonal antibody binds to interleukin-1 β and blocks the binding of interleukin-1 β to interleukin-1 receptors, and thus blocking the biological activity of interleukin-1 β . The monoclonal antibody disclosed in this patent may be obtained by production of an immunogen through genetic engineering using recombinant DNA technology. The immunogen is injected into a mouse and thereafter spleen cells of the mouse are immortalized by fusing the spleen cells with myeloma cells. The resulting cells include the hybrid continuous cell lines (hybridomas) that may be later screened for monoclonal antibodies. This patent states that the monoclonal antibodies of the invention may be used therapeutically, such as for example, in the immunization of a patient, or the monoclonal antibodies may be bound to a toxin to form an immunotoxin or to a radioactive material or drug to form a radio pharmaceutical or pharmaceutical.

U.S. Patent No. 4,766,069 discloses a recombinant DNA cloning vehicle having a DNA sequence comprising the human interleukin-1 gene DNA sequence. This patent provides a process for preparing human interleukin-1 β , and recovering the human interleukin-1 β . This patent discloses use of interleukin-1 as an immunological reagent in humans because of its ability to stimulate T-cells and B-cells and increase immunoglobulin synthesis.

U.S. No. 4,396,601 discloses a method for providing mammalian hosts with additional genetic capability. This patent provides that host cells capable of regeneration are removed from the host and treated with genetic material including at least one marker which allows

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for selective advantage for the host cells in which the genetic material is capable of expression and replication. This patent states that the modified host cells are then returned to the host under regenerative conditions. In the present invention, genetic material may be directly introduced (a) into host cells in vivo or (b) into synoviocytes in vitro for subsequent transplantation back into the patient's joints.

In spite of these prior art disclosures, there remains a very real and substantial need for a process wherein a gene encoding a truncated interleukin-1 receptor is used to resist the deleterious pathological changes associated with arthritis. More specifically there is a need for such a process where a gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor, capable of binding to and neutralizing interleukin-1 is expressed in host synovial cells in vivo.

SUMMARY OF THE INVENTION

The present invention has met the hereinbefore described need. A method of using the gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor is provided for in the present invention. This gene is capable of binding to and neutralizing interleukin-1 in vivo to substantially resist the degradation of cartilage in a mammalian host. Unlike previous pharmacological efforts, the method of this invention employs gene therapy in vivo to address the chronic debilitating effects of arthritis.

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A preferred method of using the gene coding for the truncated interleukin-1 receptor of this invention involves employing recombinant techniques to generate a cell line which produces infectious retroviral particles
5 containing the gene coding for the truncated interleukin-1 receptor. The producer cell line is generated by inserting the gene coding into a retroviral vector under the regulation of a suitable eukaryotic promoter, transfecting the retroviral vector containing the gene coding into the
10 retroviral packaging cell line for the production of a viral particle that is capable of expressing the gene coding, and infecting the synovial cells of a mammalian host using the viral particle.

More specifically, the method of using the
15 hereinbefore described gene involves introducing the viral particles obtained from the retroviral packaging cell line directly by intra-articular injection into a joint space of a mammalian host that is lined with synovial cells. The method of using the gene of this invention may be employed
20 both prophylactically and in the treatment of arthritis.

In another embodiment of this invention, a method of using the hereinbefore described gene involves infecting synovial cells in culture with the viral particles and subsequently transplanting the infected synovial cells back
25 into the joint. This method of using the gene of this invention may also be employed prophylactically and in the treatment of arthritis.

In another embodiment of this invention, a method of using the gene coding for an extracellular interleukin-1
30 binding domain of the interleukin-1 receptor that is capable of binding to and neutralizing interleukin 1 includes employing recombinant techniques to produce a retrovirus vector carrying two genes. The first gene encodes the

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extracellular interleukin-1 binding domain of the interleukin receptor, and the second gene encodes for selectable antibiotic resistance. This method of use involves transfecting the retrovirus vector into a retrovirus packaging cell line to obtain a cell line producing infectious retroviral particles carrying the gene.

Another embodiment of this invention provides a method of preparing a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor including synthesizing the gene by a polymerase chain reaction, introducing the amplified interleukin-1 receptor coding sequence into a retroviral vector, transfecting the retroviral vector into a retrovirus packaging cell line and collecting viral particles from the retrovirus packaging cell line.

In another embodiment of this invention, a compound for parenteral administration to a patient in a therapeutically effective amount is provided for that contains a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor and a suitable pharmaceutical carrier.

Another embodiment of this invention provides for a compound for parenteral administration to a patient in a prophylactically effective amount that includes a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor and a suitable pharmaceutical carrier.

It is an object of the present invention to provide a method of using in vivo a gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1, including interleukin-1 α and interleukin-1 β .

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It is an object of the present invention to provide a method of using a gene in vivo in a mammalian host that is capable of binding to and neutralizing substantially all isoforms of interleukin-1 and thus, substantially resist the degradation of cartilage and protect surrounding soft tissues of the joint space.

It is an object of the present invention to provide a method of using in vivo a gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1 for the prevention of arthritis in patients that demonstrate a high susceptibility for developing the disease.

It is an object of the present invention to provide a method of using in vivo a gene coding for an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1 for the treatment of patients with arthritis.

It is an object of the present invention to provide a method of using in vivo a gene or genes that address the chronic debilitating pathophysiology of arthritis.

It is a further object of the present invention to provide a compound for parenteral administration to a patient which comprises a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor and a suitable pharmaceutical carrier.

These and other objects of the invention will be more fully understood from the following description of the invention, the referenced drawings attached hereto and the claims appended hereto.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the interleukin-1 binding domain amino acid arrangement.

Figure 2 shows the amino acid and nucleotide sequence of the human and mouse interleukin-1 receptors.

Figure 3 shows gene encoding a truncated interleukin-1 receptor inserted into a retroviral vector.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "patient" includes members of the animal kingdom including but not limited to human beings.

The gene and method of using the gene of this invention provide for the neutralization of interleukin-1. Interleukin-1 is a key mediator of cartilage destruction in arthritis. Interleukin-1 also causes inflammation and is a very powerful inducer of bone resorption. Many of these effects result from the ability of interleukin-1 to increase enormously the cellular synthesis of prostaglandin E₂, the neutral proteinases-- collagenase, gelatinase, and stromelysin, and plasminogen activator. The catabolic effects of interleukin-1 upon cartilage are exacerbated by its ability to suppress the synthesis of the cartilaginous matrix by chondrocytes. Interleukin-1 is present at high concentrations in synovial fluids aspirated from arthritic joints and it has been demonstrated that intra-articular injection of recombinant interleukin-1 in animals causes cartilage breakdown and inflammation.

Interleukin-1 exists as several species, each an unglycosylated polypeptide of 17,000 Daltons. Two species have previously been cloned, interleukin-1 α and interleukin-1 β . The α form has a pI of approximately 5, and the β form has a pI around 7. Despite the existence of these isoforms, interleukin-1 α and interleukin-1 β have

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substantially identical biological properties and share a common cell surface receptor. The interleukin-1 receptor is a 80kDa (kilodalton) glycoprotein and contains an extracellular, interleukin-1 binding portion of 319 amino acids which are arranged in three immunoglobulin-like domains held together by disulfide bridges as shown in Figure 1. A 21 amino acid trans-membrane domain joins the extracellular portion to the 217 amino acid cytoplasmic domain. Figure 2 shows the amino acid and nucleotide sequence of the human and mouse interleukin-1 receptors. In Figure 2, the 21 amino acid trans-membrane region of the interleukin-1 receptor is marked by the solid line. The position of the 5' and 3' oligonucleotides for PCR are also marked by a short solid line. The lysine amino acid just 5' to the trans-membrane domain to be mutated to a stop codon is marked by a solid circle in Figure 2.

Synovium is by far the major, and perhaps the only, intra-articular source of interleukin-1 in the arthritic joint. Synovia recovered from arthritic joints secrete high levels of interleukin-1. Both the resident synoviocytes and infiltrating blood mononuclear cells within the synovial lining produce interleukin-1.

The present invention provides a method of using in vivo a gene coding for a truncated form of the interleukin-1 receptor which retains its ability to bind interleukin-1 with high affinity but which is released extracellularly and therefore inactive in signal transduction. The binding of this truncated and modified receptor to interleukin-1 inhibits the intra-articular activity of interleukin-1.

This method of using a gene encoding the extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and

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neutralizing interleukin-1 includes employing a retroviral vector carrying a truncated interleukin-1 receptor gene which encodes a truncated and soluble active form of the receptor. The expression of the novel interleukin-1 receptor gene is controlled by regulatory sequences contained within the vector that are active in eukaryotic cells. This recombinant viral vector is transfected into cell lines stably expressing the viral proteins in trans required for production of infectious virus particles carrying the recombinant vector. These viral particles are used to deliver the recombinant interleukin-1 receptor to the recipient synovial cells by direct virus infection in vivo.

The soluble human interleukin-1 receptor to be inserted into the retroviral vector may be generated by a polymerase chain reaction (PCR). An oligonucleotide complementary to the 5' leader sequence of the human interleukin-1 receptor (GCGGATCCCCTCCTAGAAGCT) and an oligonucleotide complementary to a region just upstream from the trans-membrane domain of the interleukin-1 receptor (GCGGATCCCATGTGCTACTGG) are used as primers for PCR. The primer for the region of the interleukin-1 receptor adjacent to the trans-membrane domain contains a single base change so that the lys codon at amino acid 319 (AAG) is changed to a stop codon (TAG). By inserting a translation stop codon just upstream from the transmembrane domain, a truncated form of interleukin-1 receptor that is secreted by the cell is generated. A BamHI recognition sequence (GGATCC) is added to the 5' end of the PCR primers, and following amplification, the resulting interleukin-1 receptor fragment is cloned into a BamHI site. A cDNA library from human T-cells is used as a source for the interleukin-1 receptor cDNA. To amplify the appropriate region of the interleukin-1

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receptor from the cDNA library, the complementary primers are added to the DNA and 50 cycles of annealing, primer extension and denaturation are performed using a thermocycler and standard PCR reaction conditions well known by those persons skilled in the art. Following amplification of the interleukin-1 soluble receptor using the PCR process, the resulting fragment is digested with BamHI and inserted into the pLJ retroviral vector. The pLJ retroviral vector is available from A. J. Korman and R. C. Mulligan. See also Proc. Natl. Acad. Sci., Vol. 84, pp. 2150-2154 (April 1987) co-authored by Alan J. Korman, J. Daniel Frantz, Jack L. Strominger and Richard C. Mulligan. Restriction analysis was performed to determine the correct orientation of the insert.

The retrovirus vector carrying the truncated interleukin-1 receptor is transferred into the CRIP (Proc. Natl. Acad. Sci., Vol. 85, pp. 6460-6464 (1988), O. Danos and R. C. Mulligan) packaging cell line using a standard CaPO_4 transfection procedure and cells wherein the viral vector is stably integrated and is selected on the basis of resistance to the antibiotic G418. The viral vector containing the neomycin resistant (neo-r) gene is capable of imparting resistance of the cell line to G418. The CRIP cell line expresses the three viral proteins required for packaging the vector viral RNAs into infectious particles. Moreover, the viral particles produced by the CRIP cell line are able to efficiently infect a wide variety of mammalian cell types including human cells. All retroviral particles produced by this cell line are defective for replication but retain the ability to stably integrate into synovial cells thereby becoming an heritable trait of these cells. Virus stocks produced by this method are substantially free of

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contaminating helper-virus particles and are also non-pathogenic.

More specifically, the truncated interleukin-1 gene can be inserted into a retroviral vector under the regulation of a suitable eukaryotic promoter such as the retroviral promoter already contained within the gene transfer vector, such as for example, the pLJ vector shown in Figure 3. It will be understood by those persons skilled in the art that other vectors containing different eukaryotic promoters may also be utilized to obtain a generally maximal level of interleukin-1 receptor expression. The vectors containing the truncated, and modified interleukin-1 receptor will be introduced into a retroviral packaging cell line (CRIP) by transfection and stable transformants isolated by selection for the expression of the neomycin resistance gene also carried by the pLJ vector. The CRIP cell line expresses all the proteins required for packaging of the exogenous retroviral RNA. Viral particles produced by the G418-selected CRIP cell lines will carry a recombinant retrovirus able to infect mammalian cells and stably express the interleukin-1 truncated receptor. The viral particles are used to infect synovial cells directly in vivo by injecting the virus into the joint space.

Another embodiment of this invention provides a method for using the hereinbefore described viral particles to infect in culture synovial cells obtained from the lining of the joint of a mammalian host. The advantage of the infection of synovial cells in culture is that infected cells harboring the interleukin-1 receptor retroviral construct can be selected using G418 for expression of the neomycin resistance gene. The infected synovial cells expressing the interleukin-1 receptor can then be

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transplanted back into the joint by intra-articular injection. The transplanted cells will express high levels of soluble interleukin-1 receptor in the joint space thereby binding to and neutralizing substantially all isoforms of interleukin-1, including interleukin-1 α and interleukin-1 β .

The method used for transplantation of the synovial cells within the joint is a routine and relatively minor procedure used in the treatment of chronic inflammatory joint disease. Although synovium can be recovered from the joint during open surgery, it is now common to perform synovectomies, especially of the knee, through the arthroscope. The arthroscope is a small, hollow rod inserted into the knee via a small puncture wound. In addition to permitting the intra-articular insertion of a fibre-optic system, the arthroscope allows access to surgical instruments, such that synovial tissue can be removed arthroscopically. Such procedures can be carried out under "spinal" anesthetic and the patient allowed home the same day. In this manner sufficient synovium can be obtained from patients who will receive this gene therapy.

The synovial cells (synoviocytes) contained within the excised tissue may be aseptically recovered by enzymic digestion of the connective tissue matrix. Generally, the synovium is cut into pieces of approximately 1 millimeter diameter and digested sequentially with trypsin (0.2% w/v in Grey's Balanced Salt Solution) for 30 minutes at 37° centigrade, and collagenase (0.2% w/v in Grey's Balanced Salt Solution) for 2 hours at 37° centigrade. Cells recovered from this digestion are seeded into plastic culture dishes at a concentration of 10^4 - 10^5 cells per square centimeter with Hank's F₁₂ medium supplemented with 10% foetal bovine serum and antibiotics. After 3-7 days,

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the culture medium is withdrawn. Non-adherent cells such as lymphocytes are removed by washing with Grey's Balanced Salt Solution and fresh medium added. The adherent cells can now be used as they are, allowed to grow to confluency or taken through one or more subcultures. Subcultivating expands the cell number and removes non-dividing cells such as macrophages.

Following genetic manipulation of the cells thus recovered, they can be removed from the culture dish by trypsinising, scraping or other means, and made into a standard suspension. Grey's Balanced Salt Solution or other isogenic salt solutions of suitable composition, or saline solution are suitable carriers. A suspension of cells can then be injected into the recipient mammalian joint. Intra-articular injections of this type are routine and easily carried out in the doctor's office. No surgery is necessary. Very large numbers of cells can be introduced in this way and repeat injections carried out as needed.

Another embodiment of this invention is the gene produced by the hereinbefore described method of preparation. This gene carried by the retrovirus may be incorporated in a suitable pharmaceutical carrier, such as for example, buffered physiologic saline, for parenteral administration. This gene may be administered to a patient in a therapeutically effective dose. More specifically, this gene may be incorporated in a suitable pharmaceutical carrier at a therapeutically effective dose and administered by intra-articular injection.

In another embodiment of this invention, this gene may be administered to patients as a prophylactic measure to prevent the development of arthritis in those patients determined to be highly susceptible of developing this disease. More specifically, this gene carried by the

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retrovirus may be incorporated in a suitable pharmaceutical carrier at a prophylactically effective dose and administered by parenteral injection, including intra-articular injection.

5 It will be appreciated by those persons skilled in the art that this invention provides a method of using and a method of preparing a gene encoding an extra cellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing substantially
10 all isoforms of interleukin-1, and thus substantially protect cartilage of a mammalian host from pathological degradation. In addition, it will be understood by those persons skilled in the art that the method of using the gene of this invention will reduce inflammation, protect soft
15 tissues of the joint and suppress the loss of bone that occurs in patients suffering with arthritis.

 It will be appreciated by those persons skilled in the art that the viral vectors employed in the hereinbefore described invention may be employed to transfect synovial
20 cells in vivo or in culture, such as by direct intra-articular injection or transplantation of autologous synovial cells from the patient transduced with the retroviral vector carrying the truncated interleukin-1 receptor gene.

25 Whereas particular embodiments of this invention have been described above for purposes of illustration, it will be evident to those persons skilled in the art that numerous variations of the details of the present invention may be made without departing from the invention as defined
30 in the appended claims.

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We claim:

1. A method of using a gene coding for a truncated interleukin-1 receptor to resist deleterious pathological changes associated with arthritis which comprises:

5 employing recombinant techniques to produce a retroviral packaging cell line which contain said gene coding for said truncated interleukin-1 receptor;

 inserting said gene coding for said truncated interleukin-1 receptor into a retroviral vector wherein said
10 retroviral vector is under the regulation of a suitable eukaryotic promoter;

 transfecting said retroviral vector containing said gene coding for said truncated interleukin-1 receptor into said retroviral packaging cell line for the production
15 of a viral particle that is capable of expressing said gene coding for said truncated interleukin-1 receptor; and

 infecting synovial cells of a mammalian host using said viral particle obtained from said retroviral packaging cell line.

2. The method of Claim 1 employing said gene having DNA that is capable of replication and expression in said synovial cells lining a joint space of said mammalian host.

3. The method of Claim 1 including employing said method to substantially prevent the development of arthritis in a patient having a high susceptibility of developing arthritis.

4. The method of Claim 1 including employing said method to treat an arthritic patient.

5. The method of Claim 1 including effecting the infection of said synovial cells of a mammalian host by

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introducing said viral particle directly into said synovial cells lining a joint space of said mammalian host.

6. The method of Claim 5 including effecting said introduction of said viral particle by parenteral injection.

7. The method of Claim 5 including effecting said introduction of said viral particle by intra-articular injection.

8. The method of Claim 1 including effecting said infection of said synovial cells of a mammalian host by introducing said viral particles directly into synovial cells in culture to form transduced synovial cells which may
5 be subsequently transplanted into a patient's joint.

9. The method of Claim 8 including effecting said transplantation of said transduced synovial cells into a patient's joint by employing intra-articular injection.

10. The method of Claim 1 including effecting said infection of said synovial cells of a mammalian host by introducing said viral particles to outer synovial cells.

11. A method of using a gene having a coding for a extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing interleukin-1 which comprises:

5 employing recombinant techniques to produce a retrovirus vector carrying two genes wherein a first gene encodes said extracellular interleukin-1 binding domain of said interleukin-1 receptor and a second gene encodes for selectable antibiotic resistance; and

10 transfecting said retrovirus vector into a retrovirus packaging cell line to obtain a cell line producing nonpathogenic, replication deficient but integration competent, amphotrophic infectious retroviral particles carrying said gene.

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12. The method of Claim 11 including initiating introduction of said gene by infection with said retroviral particles from said cell line directly into synovial cells lining a joint space of a mammalian host.

13. The method of Claim 11 including initiating introduction of said gene by transduction of autologous synovial cells in culture, selecting a synoviocyte cell line by treatment of cultures with antibiotic, and transplanting
5 said selected synoviocyte cells into an affected mammalian joint.

14. The method of Claim 11 wherein effecting said introduction of said viral particles is by parenteral injection.

15. The method of Claim 11 wherein effecting said introduction of said viral particles is by intra-articular injection.

16. A method for preparing a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to an neutralizing interleukin-1 which comprises:

5 synthesizing said gene by a polymerase chain reaction of said extracellular interleukin-1 binding domain including a signal sequence for secretion of a protein;

introducing amplified interleukin-1 receptor coding sequence into a retroviral vector;

10 transfecting said retroviral vector into a amphotrophic retrovirus packaging cell line; and

collecting viral particles obtained from said retrovirus packaging cell line, wherein said viral particles contain said gene.

17. The gene prepared by the process of Claim 16.

18. A compound for parenteral administration to a patient in a therapeutically effective amount which

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comprises a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor and a suitable pharmaceutical carrier.

19. A compound for parenteral administration to a patient in a prophylactically effective amount which comprises a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor and a suitable pharmaceutical carrier.
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FIGURE 1

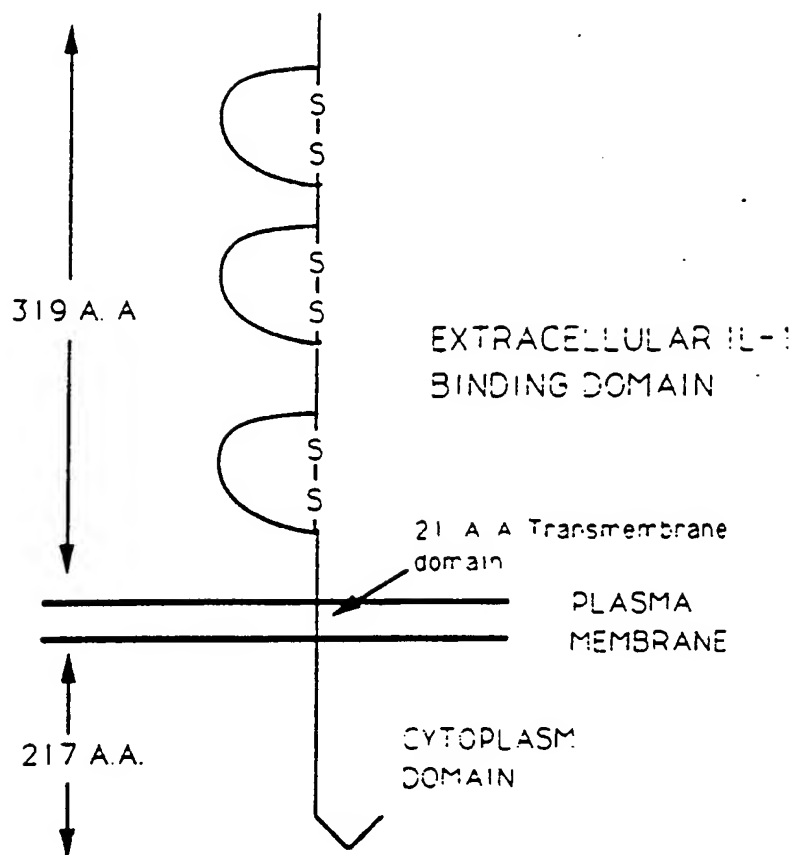


FIGURE 2

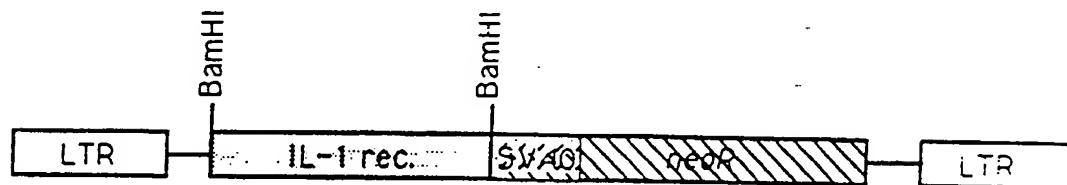
2/3

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

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FIGURE 3

Structure of the PLJ-ILrec retroviral vector
and partial restriction endonuclease map



LTR - Long Terminal Repeats - regulates viral
transcription and expression of IL-1 receptor

Neo^r - bacterial gene encoding resistance to the antibiotic
neomycin

SV40 - Simian Virus 40 enhancer promoter - regulates
expression of the neo^r gene

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09231

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): C12N 15/00; C07H 15/12; A61K 31/70 US CL : 435/172.3; 536/27; 424/548; 514/44		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	435/172.3; 536/27; 424/548; 514/44	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁵		
APS, Chem Abs, Biosis		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category*	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y	FASEB Journal, Volume 4, issued March 1990, J.E. Chin et al, "Interleukin-1 Receptors on Rabbit Articular Chondrocytes; Relationship Between Biological Activity and Receptor Binding Kinetics", pages 1481-1487, see entire document.	1-19
Y	Proceedings National Academy of Sciences, Volume 83, issued November 1986, E.R. Pettiphar et al, "Interleukin-1 Induces Leukocyte Infiltration and Cartilage Proteoglycan Degradation in the Synovial Joint", pages 8749-8753, see entire document.	1-19
Y	Journal of Immunology, Volume 141, No. 4, issued 15 August 1988, S. Banerjee et al, "Immunosuppression of Collagen-Induced Arthritis in Mice with Anti-IL-2 Receptor Antibody", pages 1150-1154, see entire document.	1-19
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents:¹⁶</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²	
12 MARCH 1992	20 MAR 1992	
International Searching Authority ¹	Signature of Authorized Officer ²⁰	
ISA/US	Deborah Crouch, Ph.D.	

BSC
(JAH NOTES)



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☐ 1: [BF786652](#) **602111409F1 NCI CGAP Kid14 Mus musculus cDNA clone** Taxonomy, LinkOut
IMAGE:4239626 5', mRNA sequence

IDENTIFIERS

dbEST Id: 7283942
EST name: 602111409F1
GenBank Acc: BF786652
GenBank gi: 12091688

CLONE INFO

Clone Id: IMAGE:4239626 (5')
Plate: LLAM9853 Row: a Column: 03
DNA type: cDNA

PRIMERS

PolyA Tail: Unknown

SEQUENCE

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CTGCTTCTTCAGAGCTCTGCAGTCTGTCTCAGGGCCTTCAGGGACCGCAGCAGCCAGC  
AACTCTCTGGTGTCTGAGTCTGTGGTGAGCTTGGCAGCCGGAACCCAGGCTGTGCTACGC  
TGCCAGAGCCCCGCATGGTGTGGACCCAAGACCGGCTGCATGATCGCCAGCGCGTGGTC  
CACTGGGACCTCAGCGGGGGCCCGGGCAGCCAACGGCGCCGACTTGTGGATATGTATTCTG  
GCGGGTGAACAGCGCGTGTACGAGCCGCGGATCGCGACCGCTCCTGCTGTGCGCTTCT  
GCTTTCCACGACGGCAACTTCTCGCTGCTCATTCGCGCTGTGGAGAGAGGCGATGAAGGG  
GTGTACACCTGCAACCTGCACCATCACTACTGCCACCTCGATGAGAGCCTGGCTGTGCGC  
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TGGTGGTGGCCCATG
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Quality: High quality sequence stops at base: 615

Entry Created: Jan 5 2001
Last Updated: Jan 12 2001

COMMENTS

Tissue Procurement: Jeffrey E. Green, M.D.
cDNA Library Preparation: Life Technologies, Inc.
cDNA Library Arrayed by: The I.M.A.G.E. Consortium (LLNL)
DNA Sequencing by: Incyte Genomics, Inc.
Clone distribution: MGC clone distribution information can
be found through the I.M.A.G.E. Consortium/LLNL at:
<http://image.llnl.gov>

LIBRARY

Lib Name: NCI_CGAP_Kid14
Organism: Mus musculus
Strain: FVB/N
Organ: kidney
Lab host: DH10B (T1 phage-resistant)
Vector: pCMV-SPORT6
R. Site 1: NotI

R. Site 2: SalI
Description: Cloned unidirectionally. Primer: Oligo dT. Average insert size 1.75 kb. Constructed by Life Technologies. Note: this is a NCI_CGAP Library. |

SUBMITTER

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Tel: (301) 496-1550
E-mail: Robert_Strausberg@nih.gov

CITATIONS

Title: National Institutes of Health, Mammalian Gene Collection (MGC)
Authors: NIH-MGC <http://mgc.nci.nih.gov/>
Year: 1999
Status: Unpublished

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PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM

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☐ 1: [AI593811](#) **vr22h10.y1 Barstead mouse myotubes MPLRB5 Mus musculus** Taxonomy, LinkOut
cDNA clone IMAGE:1121443 5', mRNA sequence

IDENTIFIERS

dbEST Id: 2412245
EST name: vr22h10.y1
GenBank Acc: AI593811
GenBank gi: 4602859

CLONE INFO

Clone Id: IMAGE:1121443 (5')
Source: IMAGE Consortium, LLNL
DNA type: cDNA

PRIMERS

Sequencing: -40RP from Gibco
PolyA Tail: Unknown

SEQUENCE

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AATTCGGATCCTTGCTGTTTTTCTGGAGAGTTAGAGCTGAGTAAGACAAAGCACGTCCCC
CGCAGGCGCCATGGAAC TGCTGTCCCGTGCTGTGGAAACTGCTGCTTCTTCAGAG
CTCTGCAGTCCTGTCTCAGGGCCTTCAGGGACCGCAGCAGCCAGCAACTCTCTGGTGTC
TGAGTCTGTGGTGAGCTTGGCAGCCGGAACCCAGGCTGTGCTACGCTGCCAGAGCCCCCG
CATGGTGTGGACCCAAGACCGGCTGCATGATCGCCAGCGCGTGGTCCACTGGGACCTCAG
CGGGGACCCGGGCAGCCAACGGCGCCGACTTGTGGATATGTATTTCGGCGGGTGAACAGCG
CGTGACGAGCCGCGCGATCGCGACCGCCTCCTGCTGTGCGCTTCTGCTTTCACGACGG
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CTGCACCATCACTACTGCCACCTCGATGAGAG
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Quality: High quality sequence stops at base: 476

Entry Created: Apr 12 1999
Last Updated: Apr 21 1999

COMMENTS

This clone is available royalty-free through LLNL ; contact the IMAGE Consortium (info@image.llnl.gov) for further information.
MGI:610779
This read is a RESEQUENCE of a previously sequenced mouse clone
This read has been verified (found to hit its original self in the correct orientation)

LIBRARY

Lib Name: Barstead mouse myotubes MPLRB5
Organism: Mus musculus
Strain: C3H
Cell line: C2C12
Lab host: DH10B
Vector: pT7T3D-Pac (Pharmacia) with a modified polylinker
R. Site 1: EcoRI

R. Site 2: NotI
Description: 1st strand cDNA was primed with a Not I - oligo(dT) primer [5' TGTTACGAATCTGAAGTGGGAGCGGCCGCCCTTTTTTTTTTTTTTTTTTTTTTTT 3']; double-stranded cDNA was ligated to Eco RI adaptors [AATTCGGATCCTTG], digested with Not I and cloned into the Not I and Eco RI sites of the modified pT7T3 vector. Library constructed by Bob Barstead. The C2C12 cell line (available from ATCC, catalog # CRL-1772) differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins.

SUBMITTER

Name: Marra M/WashU-NCI Mouse EST Project 1999
Institution: Washington University School of Medicine
Address: 4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA
Tel: 314 286 1800
Fax: 314 286 1810
E-mail: mouseest@watson.wustl.edu

CITATIONS

Title: The WashU-NCI Mouse EST Project 1999
Authors: Marra,M., Hillier,L., Kucaba,T., Martin,J., Beck,C., Wylie,T., Underwood,K., Steptoe,M., Theising,B., Allen,M., Bowers,Y., Person,B., Swaller,T., Gibbons,M., Pape,D., Harvey,N., Schurk,R., Ritter,E., Kohn,S., Shin,T., Jackson,Y., Cardenas,M., McCann,R., Waterston,R., Wilson,R.
Year: 1999
Status: Unpublished

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